#### Altered Tost Purctor Evolved Ligand Binding

Plasmid encoding Ag + CD28BP

APC CD28BP

TCR CD28(T cell) CTLA-4

T cell activation

Plasmid encoding Ag + CTLA-4BP

APC CTLA-4BP

CD28 (T cell) CTLA-4

T cell anergy/ tolerance

MA YGEN

MGHTRRQGTSPSKCPYLNPPQLLVLAGLSHFCSG- -VIHVTKEVKSVATLSCGHNVSVBELAQTRIYRQKEKKMVLTMMS MGHTMKWGSLPPKRPCLWLSQLLVLIGLFYFCSGITPKSVTYRVKETVMLSCDYSTSTBELTSLRIYNQKDSYNALLDP MGHTMKWGSLIPPKRPCLMLSQLLVLTGLFYFCSGTTPKSVTRRVKETVMLSCDYNTSTERLTSLR1YWQKDSKWVLALLP MGHTMKWRSLPPKRPCTMLSQLLVTJIGLPYPCSGTTPKSVTKRVKETVMLSCDYSTSTERLIFSLRLYWQXDGKMVLATLP MGHTMKNGSLIPPKRPCLMLSQLLVL/YGLFYFCSGTTPKSVTKRVKETVMLSCDYSTSTEEL/TSLR1YNQXOSYAWLALLD MGHTLRPGTPLPRCIHIKUCULIALAGIHFSSG---ISQVTKSVKEMAALSCDYNISIDELANMIYWQXDQQMVLSIIS MGHTMKWGSLPPKRPCLMLGQLLVLTGLFYPCSG1TPKSVTKRVKETVMLSCDYNTSTBELLTGLR LYWQKDGKMVLA L LP MGHTMKNGSLIPPKRPCLMLSQLLVLTGLFYFCSGTTPKSVTXRVKGTVMLSCDYNTSTKELLTSLRIYWQXOSX0VLATLIP MGHTMKWGSLPPKRPCLMLSQLLVLTGLPYPCSGTTPKSVTXRVKGTVNLSCDYNTSTRELJTSLRIYMQXOSKOVLALLP MGHTMKWGSLIPPKRPCLWLGQLLVLTGLFYPCSG1TPKSVTXRVKGTVMLSCDYNTSTEELTSLR1YWQXDSKWVLALLP MGHTMKWGSLIPPKRPCLMLSQLLVLIVLISHPY FCSGLTPKSVTKRVKBTVMLSCDYSTSTBELTSLR1YMQKDSROVLALLP MGHTLRRGFPLPRCLHLKLCLLLALAGLHFSSG---ISQVTKSVKEMAALSCDYNISIDELARMRIYWQKDQQMVLSIIS MGHTLRPGTPLPRCLHLKLCLLLLALAGLHPSSG---ISQVTKSVKEMAALSCDYNISIDELARMRIYWQXDQQMVLSIIS MGHTMKWGSL PPKRPCLINLSQLLVLTGLFY FCSGI TPKSVTKRVKETVMLSCDXSTSTRBLTSLRI YWQKDSKMVLAILP MGHTWKWGSLDPKRPCLWLSQLLVLTGLFYFCSGI TPKSVTKRVKETVMLSCOYSTSTEELTSLRI Y4QKDSKMVLAILD MGHTMXWGSLPPKRPCLMLSQLLVLTGLFYFCSGTTPKSVTKRVKETVMLSCDYNTSTBELTSLR1YMQXDSKMVLAILP MGHTMKMGSLPPKRPCLMLSQLLVLTGLFYPCSGITPKSVTKRVKETVMLSCDYNTSTEELISLRIYWQKDSKMVLAILP MCHTMKWGSLPPKRPCLWLSQLLVLTGLFYFCSGITPKSVTKRVKETVMLSCDYSTSTEELTBLRIYWQKDSKMTAILP MGHTMKWRSLPPKRPCL#LSQLLVLTGLFYFCSGTTPKSVTKRVKETVMLSCDYNTSTERLTSLR1YWQXDSKMVLAILP MGHTMKMGSLPPKRPCEMLSQLJVLTGLFYFCSGTTPKSVTKRVKETVMLSCDYNTSTEELIFSLR1YMQKOSKMVLA.LLP MGHTMKNGSLPPKRPCLWLSQLLVLTGLPYFCSGLTPKSVTKRVKETVMLSCDYNASTEELTSLR I YWQKDSKWVLA I LP MGYTMKNGSLPPKRPCLMLSOLLVLTGLPYPCSGTTPKSVTKRVKBTVMLSCDYSTSTEELTSLR1YWOKDSKMVLA1LP MGHTMKWGSLEPKRPCLWLSQLLVLTGLPYRCSGITPKSVTKRVKBTVMLSCDYSTSTBELTSLRIYWQKDSKWVLAILP MGHTMKWGSLPPKRPCL#LSQLLVLTGLFYFCSGTTPKSVTKRVKETVMLSCDYNTSTBELTSLR1YWRKDSKMXLATLP MGHTMKMGSLPPKCPCLMLSQLLVLIGLPXPCSQITPKSVTKRVKETVMLSCDYNTSTBELTSLRIYNQKDSKMVLAILP MGHTMKWGSLEPKRPCLWLSQLLVLTGLEYFCSGITPKSVTKRVKBTVMLSCDYNTSTBKLTSLR1YWQKDSKMVLAILP MGHTMKWGSLIPPKRPCLMLSQLLVLTGLFYFCSGTTPKSVTXRVKBTVMLSCDYNTSTBELTSLRTYMQKOSKMYLATLP MGHTMKMGSLIPPKRPCLMLPQLLVT/GLFYFCSG1TPKSVTXRVKFTWLSCDYNTSTBELT/SLA1YMQKOSKWVLA1LP MGHTMKRIGSLIFFKRPCLMLSQLLVLIGLFYPCSGTTPKSVTKRVKETVMLSCDYNTSTFELLISLRIYMQKOSKMVLATLP MGHTMKWGSLEPKRPCLMLSQLLVLTGLEYPCSGITPKSVTKRVKBTVWPSCDYSTSTBELTSLRIYNQKOSKNVLAILP MGHTWIKWGSLDPRRPCLWLSQLLVLTGLPYPCSGI TPKSVTKRVKBTVWLSCDYNTSTEBLTSLR I YWQXDSKWNLAI LP MGHTMINGSLIPPINPCLMLSQLLV1,TGLPYFCSGLTPKSVTTRRVIRPTWLSCDYSTSTFEL/TSLR1 YMQIOSIONVLA I LIP MGHTMIGRGSLIPPKRPCLMPSQLLVL/TGLFYPCSG1TPKSVTXRVKGTVMLSCDYNTSTRELITSLR1YMQKOSROVLA1LP MGHTMKMGSLPPKRPCLWLSQLLVL,TGLFYFCSGTTPKSVTKRVKETVMLSCDYNTSTBELTSLR1YWQKDSKMVLATLP MGHTMKWGSLPPKRPCLMLSQLLVLTGLPYPCSGITPKSVTKRVKETVMLSCDYNTSTERLTSLRIYWQKDSKMVLAILP Extracellular domain (ECD) -Signal sequence -3  $\Xi$ 3 33  $\Xi$ 3 2222222222 SEQ: 052 R2 CD28BP-1 SEQ: 053 R2 CD28BP-2 SEQ: 054 R2 CD28BP-3 SEQ: 055 R2 CD28BP-4 SEQ: 056 R2 CD28BP-4 SEQ: 057 R2 CD28BP-6 SEQ: 057 R2 CD28BP-6 SEQ: 059 R2 CD28BP-7 SEQ: 069 R2 CD28BP-8 SEQ: 060 R2 CD28BP-8 SEQ:049\_R1\_Clone\_71 SEQ:049\_R1\_Clone\_84 SEQ:050\_R1\_Clone\_118 SEQ:051\_R1\_Clone\_126 SEQ: 061\_R2\_CD28BP-10 SEQ: 062\_R2\_CD28BP-11 SEQ: 063\_R2\_CD28BP-12 SEQ: 064\_R2\_CD28BP-13 SEQ:065\_R2\_CD28BP-14 SEQ:066\_R2\_CD28BP-15 SEQ:067\_R2\_CD28BP-16 SEQ:068\_R2\_CD28BP-17 SEQ:174 cd28A12-5 SEQ:175\_cd28a4-5star SEQ:176\_cd28A4-9 SEQ:177\_cd28A6-9 SRQ:178\_cd28A6-1 SRQ:179\_cd28A9-4 SEQ:180 cd28A8-6 SEQ:181\_cd28B2-8 SEQ:182\_cd28B4-3 SEQ:183 cd28B6-3 SRQ:184 cd28b6-6 SE0:185 cd28b8-5star SEQ:186 cd28c11-5 SBQ:278\_Human\_B7-1

Fig. 2A

1.1 I

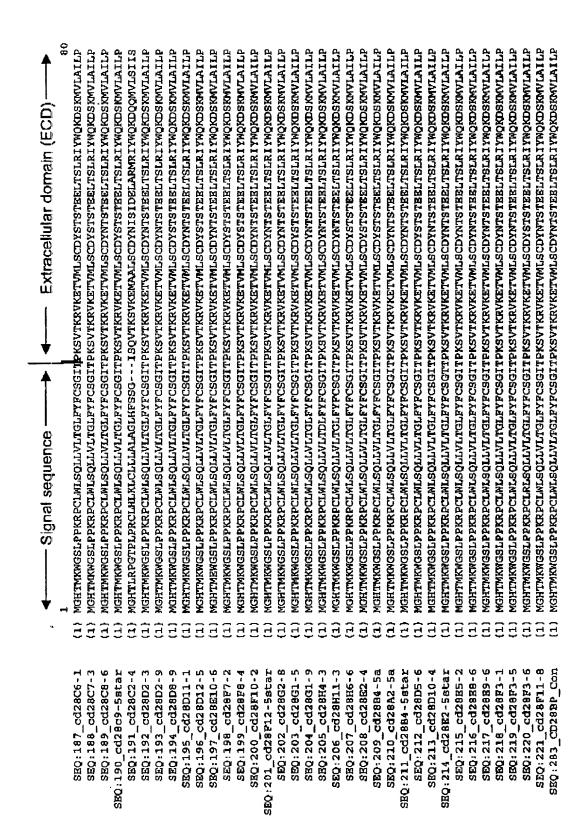


Fig. 2B

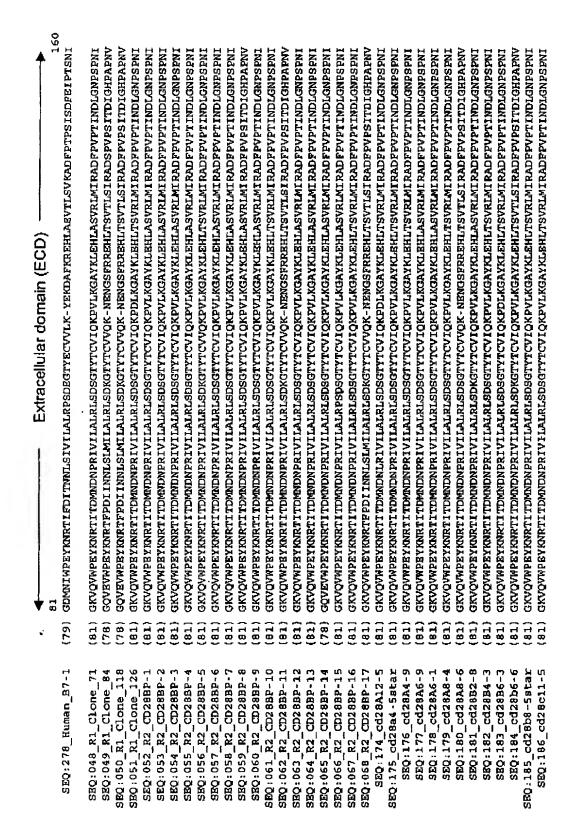


Fig. 2C

GKVQVMPRYKNRTITDMNDNPRIVILALRLSDSGTYTCVVQK-NENGSFRREHLTSVTLSIRADFPVPSITDIGHPAPNV GKVQVWPBYKNRTI TDMNDNPRI VI LALRLSDSGTYTCV I QKPVLKGAYKLEHLASVRLMI RADFPVPTINDLGNPSPNI GKVOVWPEYKNRTI TDMNDNPRI VI LALRLSDKGTYTCV I QKPVLKGAYKLEHLASVRLMI RADFPVPTINDLGNPSPNI GKVQVWPBYKNRTI TDYMDNPRI VI LALRLSDSGTYTCVI QKPVLKGAYKLEHLASVRLMI RADFPVPTINDLGNPSPNI GKYGYWPBYKNRTI TDYNDNPRI VI LALRLSDSGTYTCVI QKPDLKGAYKLRHLASVRLMI RADFPVPSI TDIGHPAPNV GKVQVMPRYKNRTI TDMNDNPRI VI LALRLSDKGTYTCVVQKPDLKGAYKLBHLAGVRLMI RADFPVPSITDIGHPAPNV gkygym pryknrti tdmndnpri vi lalrlsdsgtytcy i qkpylkgay klæhltsvrlmi radppvptindlon pspni GKVQVWPBYKNRTITDAMDNPRIVILALRLSDKGTYTCVVQK-NENGSFRRBHLTSVTLSIRADFPVPSITDIGHPAPNV gkvqvwpbyknrti tdmndnpr i v1lalrpsdsgtytcv i okpvlkgayklæhlasvrimi radfpvpt i ndlgnpspn i GKVQV#PBYXNRTTTDMNDNPRIVILALRLSDSGTYTCVIQKPDLKGAYKLEHLASVRL#IRADFFVPTINDLGNPSPNI GKVOVWPEYKNRTI TDMNDNPRI VII.ALRLSDSGTYTCVI QKPVLKGAYKLRHLTSVRLMI RADFPVPTINDLGNPSPNI GKYQVWPRYKNRTI TDMNDNPRI VILALRLSDKGTYTCVIQKPDLKGAYKLEHLASVRLMI RADFFVPTINDLGNPSPNI gkyduwprykurti tdmadnpri VI lalrlsdsgtytcvi qkedlkgayklehli svrlmi radfevpti ndlgnpspui GKVQVMPRYKNRTI TDMNDNPRIVI LALRLSDSGTYTCV IQKPDLKGAYKLEHLTSVRLMI RADFPVPTINDLGNPSPNI GKVQVMPRYKNRTI TDMNDNPRIVILALRLSDSGTYTCVIQKPVLKGAYKLEHLTSVRLMIRADFPVPTINDLGNPSPNI gkvovadrykneti tdandnprivilalrlsdsgtytcviokpvlkgayklehlasvrimiradppvptindlgnpspni GKYQVMPBYKNRTITDANDNPRIVILALRLSDSGTYTCVIQKPVLKGAYKLBHLASVRLMIRADFPVPSITDIGHPAPNV GKVQVMPEYKNRTI TDMNDNPRI VILALRLSDSGTYTCVIQKPDLKGAYKLEHLTSVRLMI RADFPVPSITDIGHPAPNV gxvqvmpbyknrtfpdi innlslmllalrlsdxgtytcvvqk-nengsfrrehltsvtlstradfpvssitdighpapnv gkvovapeyknrti tomnonpri vi lalrisdsgtytcv i okpylkgayklehltsvrimi radppvps i to i ghpapnv GKVQVMPRYKNRTI TDMNDNPRI VI LALRLSDSGTYTCV I QKPV LKGAYKLBHLASVRLMI RADFPVPTINDLGNPSPNI gkvovyprykortitdmadnprivilalrlsdsgtytcviqkpylkgayklbhlasvrlmiradffvpytindlgnpspni **GKYOVWPEYKNRTITDMINDNPRLVILALRLSDSGTYTCVIQKPVLKGAYKLBHLASVRLMIRADFPVPTINDLGNPSPNI** GKVQVNPEYKNRTITDMNDNPRIVILALRLSDSGTYTCVIQKPDLKGAYKLEHLTSVRLMIRADFPVPSITDIGHPAPNV GKVQVNPEYKNRTITDMNDNPRIVILALRLSDSGTYTCVIQKPVLKGAYKLBHLTSVRLMIRADFPVPTINDLGNPSPNI gkvonpeyknrti tomndnpri vi lalrlsdsgtytcvvok-nrngsprrehltsvtlsi radfpvpsitdighpapnv GKYQVWPEYKNRTITDANDNPRIVILALRLSDSGTYTCVIQKPVLKGAYKLBHLASVRLMIRADFPVPTINDLGNPSPNI gkvovmpryknrti tdandnpr i vi lalalgdsgtytcv i okpolkgayklahlasvrlal radfpvps i to i ohpapnv **GKYQVWPEYKNRTITDMWDNPRIVILALRLSDSGTYTCVIQKPVLKGAYKLEHLASVRLMIRADFPVPTINDLGNPSPNI** GKVQVHPBYKNRTITDMADNPRIVILALRLSDSGTYTCVIQKPVLKGAYKLEHLASVRLMIRADFPVPTINDLGNPSPNI gqvbvnpbyknrtitidandnprivilalrlsdsgtytcviqxpvlxgaykpehlasvrlmiradfpvptindlgnpspni GKVQVNPRYKNRTITDANDNPRIVIOALRLSDSGTYTCVIOKPVLKGAYKLEHLASVRLMIRADFDVP--TDLGNPSPNI gkvovmpeyknrtitdmndnpri vilalrisdsgiytcviokpvikgayklehltisvrimiradfevptindlgnpspni GKYQYMPBYKNRTITDMNDNPRIVILALRLSDSGTYTCVIQKPVLKGAYKLBHLASVRLMIRADFPVPTINDLGNPSPNI GKYOVW FEYKYRTI TDMNDN PRIVILALRLSDSGTYTCV I QKPVLKGAYKLBHLASVRLMI RAD FPV PTINDLGN PSPNI gkvovmpeykoktitdmandnprivilalrlsdsgtytcviokpvlkgayklæhlasvrlairadfpvpsindlgnespni Extracellular domain (ECD) (81) (81) (81) (81) (18) 81) (81) (78) 81) (18) (81) (81) (18) (01) (81) 917 81) 81) (81) (81) (81) 81) 61 81) 81) (81) 81) [81] (81) (81) (81) (81) 81) SRQ:195\_cd28D11-1 SRQ:196\_cd28D12-5 SRQ:197\_cd28E10-6 SRQ:199\_cd28F7-2 SRQ:199\_cd28F9-4 SRQ:200\_cd28F10-2 SEQ:191\_cd28C2-4 SEQ:192\_cd28D2-3 SEQ:193\_cd28D2-9 SEQ:194\_cd28D8-9 SEQ:217\_cd28E9-6 SEQ:218\_cd28E3-1 SEQ:219\_cd28E3-5 SEQ: 202\_cd28G2-8 SEQ: 203\_cd28G1-5 SEQ:207\_cd28H6-6 SEQ:208\_cd28E2-4 SEQ:216\_cd28B9-6 SEQ: 221\_cd28F11-8 SEQ:283\_CD28BP\_Con SEQ: 204\_cd28G1-9 SEQ: 205\_cd28H4-3 SBQ:206\_cd28H11~3 SEQ: 209\_cd2884-5a SEQ: 210\_cd28A2-5a SEQ:211\_cd28B4-5star SE0:201 cd28F12-5star SEQ:212\_cd28D5-6 SEQ:213\_cd28D10-4 SEQ:214 cd28E2-5star SEQ:215\_cd28E5-2 cd28F3-6 SEQ:187\_cd28C6-1 SEQ:188\_cd28C7-3 SEQ:189\_cd28C8-6 SEQ:190 cd28c9-5star SEQ:220

Fig. 2D

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Fig. 2E

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Fig. 2F

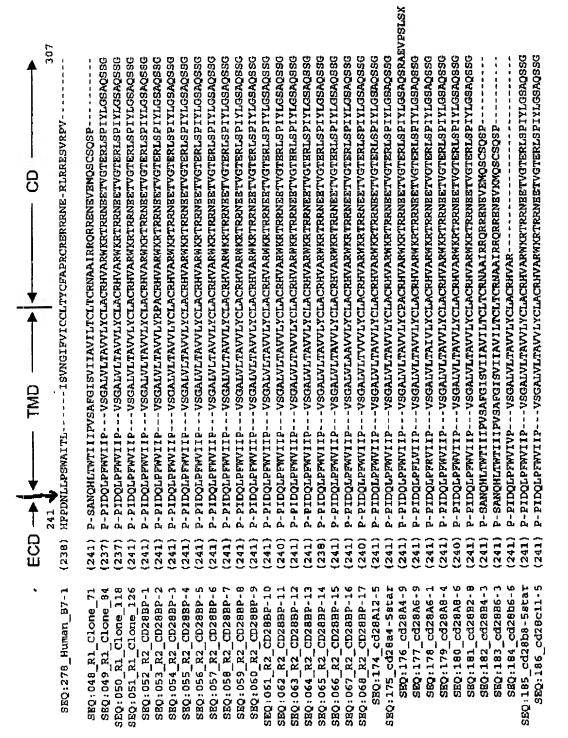


Fig. 2G

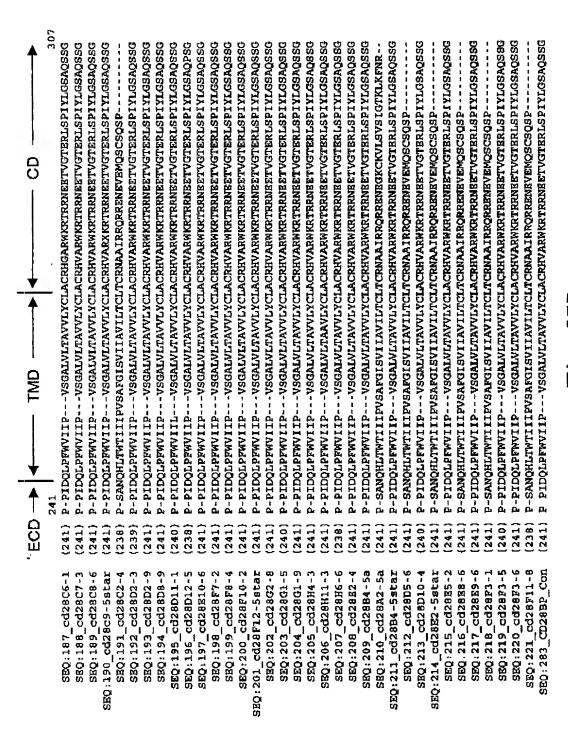
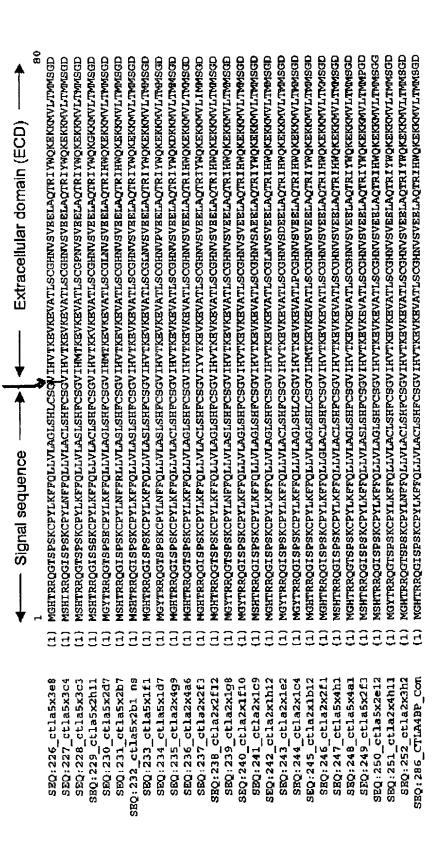


Fig. 2F

MGHTRRQGTSPSKCPYLKFFQLLVLAGLSHFCSGVIHVTKEVKEVATL9CGRNVSVBELAQTRIHWQKEKKMYLTMMSGD MSHTRRQGTSPSKCPYLKFPQLLVLAGLSHLCSGVIHVTKEVKEVATLSCGHNVSVEBLAQTRIHMQKEKKMVLTMM9GD MGYTRRQGTSPSKCPYLKFRQLLVLAGLSHLCSGVIHVTNEVKEVATLSCGHNVSGEELAQTR1YWOKEKMVLTMMYGD MGHTRRQGTSPSKCPYLKFFQLLVLACLSHPCSGV1HVTKEVKEVATLSCGHNVSVBELAQTR1HWQKEKKMVLTMMSGD MGYTRRQGTSPSKCPYLKFFQLLVLACLSHFCSGVIHVTRBVKGVATLSCGHNVSVBELAQTRIHWQKEKHONVLTMMSGD MSHTRRQGISPSKCPYLNFFRLLUVLASLSHFCSGVIHVTKEVKEVATLSCGHNVSVBELAQTRIHQKEKYOVLTMMSGD MGYTRROGISPSKCPYLKFFOLLVLACLSHFCSGVIHVTKBVKEVATLSCGHNVSVBELAQTRIYWKKKYVLTMMSGD MGYTRRQGISPSKCPYLNFPQLLVLAGLSHFCSGV1HVTXBVKGVATLSCGRNVSVRBLAQFRIYMCKGKOKVLTMMSGD MGHTRROGISPSKCPYLKFPOLLVLACLSHLCSGVIHVTKEVKEVATLSCGLNVSVBELAOTRIHMOKEKKMVLTMMSGD MGHTRROGISPSKCPYLKPFOLLVMACLSHFCSGVIHVTKBVKBVATLSCGHNVSVBELAOTRIHWOKEKKMVLTMMSGD MGHTRROGISPSKCPYLKFFQLLVLAGLPHLCSGVIHVTKEVKBVATLSCGHNVSVBELAQTRIHMQKEKKMVLTMMSGD MGHTRROGI SPSKCPYLNFFOLLVLACLSHFCSGVIHVTKEVKGVATLSCCHNVSVEBLAQTRIHWOKBKKOAVLTMMSGD MGHTRROGTSPSKCPYLMFFOLLVLACLSHFCSOVIHVTKEVKBVATLSCGHNVSVBBLACTRIHMOKBKKMVLTMMSOD MGYTRRQGTSPSKCPYLKFFQLLVLASLSHFCSGVIHVTKEVKBVATLSÇGHNVSVBBLAQTPIYMQKBKKMVLTMMSGD MGHTRROGTSPSKCPYLNPFOLLVLAGLSHPCSGVIHVTKEVKEVATLSCGHNVSVEELAQTRIYWOKBKKMVLTMMSGD MGHTRROGISPSKCPYLKPFQLLIVLAGLSHPCSGVIHVTKEVKEVATLSCGHNVSVEELAQTRIHQKEKKMVLTMMSGD MSHTRROGTSPSKCPYLK FFQLLVLASLSHFCSGV IHVITKBVKEVATLSCGHNVSVEELAQTR I YWQKEKKMVLITMISGD MGHTRROGI.SPSKCPYLKFFOLLVI.ACLSHFCSGVI.HVTKBVKEVATI.SCGHNVSVEELAOTRI.YWOKBKKMVI.TMMSGD MSHTRRQGISPSKCPYINPFQLLVLASLSHFCSGVIHVTKEVKEVATLSCGLNVSVBELAQTRIYWQKEKKMYLTMASGD MOHTRRQGISPPKCPYLNPFQLLVLACLSHFCSGVIHVTKBVKEVATLSCHNVSVRELAQTRIHMQKEKKMVLTMMSGD mshtrrogispskopylkffollvllacishfcsgvihvtkevkevatlscomnvsveelaotrihmokexkkmvltmmsdd mshtrrogispskopylkffollulasishfosguihutkrukkraatisoognrusveelaotrihmokeknaulmmsdd MSHTRROGTSPSKCPYLKPFOLLVLASLSHFCSGVIHVTKBVKEVATLSCGLNVSVBELAOTRIYMQKEKKNVLTMMSGD mshtrrogispskcpylkfpollvlaslshfcsgvihmtkgvkgvatlscghnvsvbelaotriymokekkmyltmmsgd MSHTRRQGISPSKCPYLKPFQLLIVIACLSHFCSGVIHVTKBVKEVATL8CGHNVSVBELAQTRIYMQKEKMYVLTMMSGD MGHTRRQGISPSKCPYLKFPQLLVLACLSHPCSGVIHVTKBVKEVATLACGLNVSVRELAQTRIHAQKEKKMVLTMMSGD MSHTRROGTSPSKCPYLKFPQZLVLASLSHFCSGVIHVTKEVKRVATLSCGLNVSVBBLAQTR1YMOKGKKMVLTMMSGD MGYTRROGI SPSKCPYLKFPOLLVLASLSHFCSGV I HVTKKVKBVATLSCGHNVSVBBLAQTR I HMOKBKKMVL TMMSGD Extracellular domain (ECD) Signal sequence (1) 3 3  $\Xi$  $\Xi$ 233 2222 3333 33  $\Xi$ Ξ SEQ: 074\_R2\_CTLA4BP-5×2-10c SEQ: 075\_R2\_CTLA4BP-5×2-11d SEQ: 076\_R2\_CTLA4BP-5×2-12F SEQ: 077\_R2\_CTLA4BP-5×2-2g SEQ: 078\_R2\_CTLA4BP-5×2-3c SEQ: 080\_R2\_CTLA4BP-5×2-4c SEQ: 080\_R2\_CTLA4BP-5×2-7b SEQ: 081\_R2\_CTLA4BP-5×2-7b SEQ: 081\_R2\_CTLA4BP-5×3-10e SEQ: 083\_R2\_CTLA4BP-5×3-10e SEQ: 084\_R2\_CTLA4BP-5x3-6f SEQ: 085\_R2\_CTLA4BP-5x4-11d SEQ: 087\_R2\_CTLA4BP-5x4-12c SEQ: 087\_R2\_CTLA4BP-5x4-1f SEQ: 089\_R2\_CTLA4BP-5x5-6e SEQ: 090\_R2\_CTLA4BP-5x5-6e SEQ: 090\_R2\_CTLA4BP-5x5-6e 380:092 R2 CTLA4BP-5x9-12c SE0:222 ctla5x9d10 SEQ:223\_ctla5x6f6 SEQ: 278 Human B7-1 SEQ:069 R1 CTLA4BP-5 SEQ:070 R1 CTLA4BP-7 SRQ: 071\_R1\_CTLA4BP-11 SEQ: 072 RI CTLA4BP-13 SEQ: 073 R1 CTLA4BP-27 SEQ: 224 ctla5x5h12

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7 1 11

Fig. 3B

MAIMPEYKKRTI FDITANNESIVILALRPSDEGTYECVVLKYEKDAPKREHLAEVMLSVKADPPTPSITDFRIPTSNIRRI MNIWPEYKNRTIFDITNNLSIVILALRSSDEGIYECVVLKYEKDAFKRBHLAEVTLSVKADFPTPSITDFEIPPSNIRRI MNIWPEYKNRTIFDITINNLSIVILALRPSDEGTYECVVLEYEKDAFKRBHLAEVMLSVKADFPTPSISDFBIPTSNIRRI MNIWPEYKNRTI FOITINNLSIVILALRPSDEGTYECVVLKYDKDAFKRRHLAEVTLSVKADPPTPSISDPEIPPSNIRRI MNIWPEYKNRIIFDITUNLSIVILALRPSDEGTYECVVLKYEKDAFKREHLAEVMLSVKADPPIPBISDPEIPPSNIRRI MNIWPEYKARTIFDITANLSIVILALRPSDEGTYECVVLKYEKDAFKRBHLAEVMLSVKADPPIPSISDFEIPTSNIRRI mniwprynyfifditnnlsivilalrpsdegtyecvvlkyekdafyrrhllaevmlsvkadpptesitdfæippsnirri MAI WEBYKARTI FOITUNLSIVILALR PSDBGIYECVVLKY EKDAFKREHLAFUMLSVKADP PIPS I TDFEI PPSNIRR I minpryknytifdithnisivilalrpsdegtyecvvlkyekdafkrehlaevmisvkadpptpsisdfeippsnirri mniwpeykurtifditnnlsivilalrpsdegtyecvvikyekdafkrehlaevmlsvkadpptpsitdfeippsnirri MNIWPEYKNRTI FDITUNLSIVILALRPSDEGTYECVVLKYBKDAFKREHLAEVMLSVKADPPTPSISDFRIPTSNIRRI MNIWPEYKNRTI FDITUNLSI VILLALRPSDBGTYECVVLBYEKDAPKREHLARVMLSVKADPPTPSI SDFBI PPSNIRRI MILMPEYKNRTI FDITNNLSIVILALRPSDEGTYECVVLKYEKDAFKQEHLAEVMLSVKADFPTPSITDPEIPPSNIRRI mnjwpeyknrti foltnnlsi villalrpsdegtyecvvleyekdafykrehlaevtlsykadfptpbi sdfrippsnirri MNIWPEYKNRTI FDITUNLSIVILALRPSDEGTYECVVLKYEKDAFKRBHLASWLSVKADFPTPSISDFEIPPSNIRRI MNIWPBYKKRTIFDITNNLSIVILALRPSDBGTYECVVLKYEKDAFKREHLAEVMLSVKADPPTPSISDFEIPTSNIRRI MAIWPRYKNRTIFDITNALSIVILALRPSDBGTYBCVVLKYEKDAFKRBHLAEVMLSVKADPPTP9ITDFEIPPSNIRRI miwpryknrtifdithnisivilalrpsdegtyecvvlkyekdafkrehlaevmlsvkadfptpsitdfeifppsnirri matworykantifottaalsivilalrpsdegtyecvvleyekdafkrehlaevalsvkadfftbisdfrippsnirri MIIMPEYKORTI FOLTUNLSIVILALRPSDEGTYECVVLEYEKDAFKREKLAEVTLSVKADFF1PSITDFEIPPSNIRRI MNIMPEYKNRTIFDITNMLSIVILALRPSDEGTYECVVLKYEKDARKREHLAEVTLSVKADFPTPSISDFEIPTSNIRRI MNIWPEYKKRTI FDITUNLSIVILALRPSDEGTYECVVLKYEKDAFKREHLAEVMLSVKADFPTPSITDFEI PPSNIRRI mniwpeyknrtifditnnlsivilalrpsdegtyecvvlkyekdarkrehlabytlsvkadpptpsitdfeippsnikri MYIWPEYKNRTI FDITUNLSIVILALRPSDEGTYECVVLKYEKDAFKREHLAEVTLSVKAGFPTPSITDFBIPPSNIRRI MNIWPEYKNRTIFDITNNLSIVILALRPSDEGTYECVVLKYEKDAFKREHLAKVTLSVKADFPTSISDFEIPPSNIRRI MNIWPEYXXXTI FDITNNLSIVILALRPSDEGTYECVVLKYBXDAFKREHLAEVMLSVKADPPTPSISDFRIPPSNIRRI MN IWPEYKNRTI FDI TNNLS IVILALRPSDEGTYECVVLKYEKDAFKREHLABVMLSVKADFPTPSI TDFRI PPSNI RRI MNIWPEYKKRTI FDITKNKLSIVILALRPSDEGTYECVVLKXBKDAPKREHLARVMLSVKADFPTPSITDFEIPPSNIRRI MNIMPEYKNRTIFDITNNISIVILALRPSDEGTYECVVLKYBKDAPKREHLABVTLSVKADFPTPSISDFRIPTSNIRRI Extracellular domain (ECD) (81) (81) (81) (81) (18) (18) (81) (81) (81) 81} (81) 81} (81) (81) (81) (81) (81) (81) (81) (81) (81) (81)(81) [81) 81) (81) (81) 81) SEQ: 085\_R2\_CTLA4BP-5x4-11d SEQ: 086\_R2\_CTLA4BP-5x4-12c SEQ: 087\_R2\_CTLA4BP-5x4-1f SEQ: 089\_R2\_CTLA4BP-5x5-2e SEQ: 089\_R2\_CTLA4BP-5x5-6e SEQ: 090\_R2\_CTLA4BP-5x6-9d SEQ: 091\_R2\_CTLA4BP-5x8-1f SEQ: 092\_R2\_CTLA4BP-5x8-1f SEQ: 077 R2 CTLA4BP-5x2-29 SEQ: 078 R2 CTLA4BP-5x2-40 SEQ: 080 R2 CTLA4BP-5x2-40 SEQ: 080 R2 CTLA4BP-5x2-75 SEQ: 081 R2 CTLA4BP-5x2-80 SEQ: 082 R2 CTLA4BP-5x3-10e SEQ: 083 R2 CTLA4BP-5x3-11b SEQ: 084 R2 CTLA4BP-5x3-11b SEQ:074\_R2\_CTLA4BP-5x2-10c SEQ:075\_R2\_CTLA4BP-5x2-11d SEQ:076\_R2\_CTLA4BP-5X2-12F SEQ: 223\_ctla5x6f6 SEQ: 224\_ctla5x5h12 SEQ: 225\_ctla5x5c10 SEQ: 069\_R1\_CTLA4BP-5 SEQ: 070\_R1\_CTLA4BP-7 SEQ: 071\_R1\_CTLA4BP-11 SEQ: 222\_ctla5x9d10 SEQ: 278 Human 87-1 CTLAABP-13 SE0:073 R1 CTLA4BP-27 SEQ:072 R1

Fig. 3C

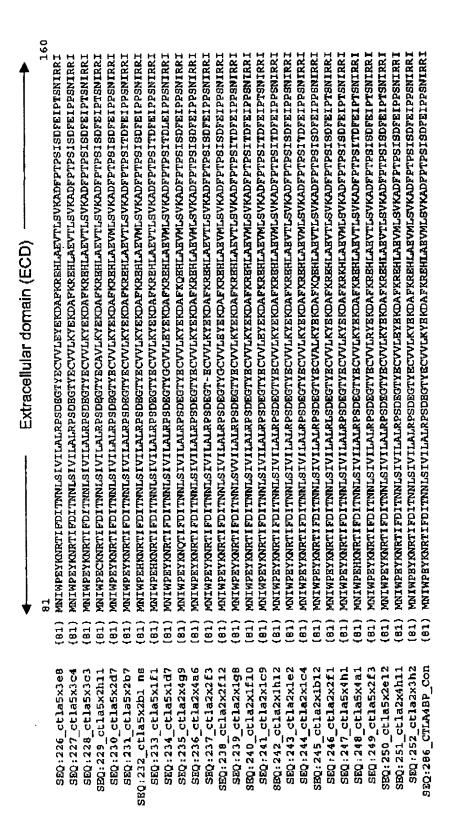


Fig. 3D

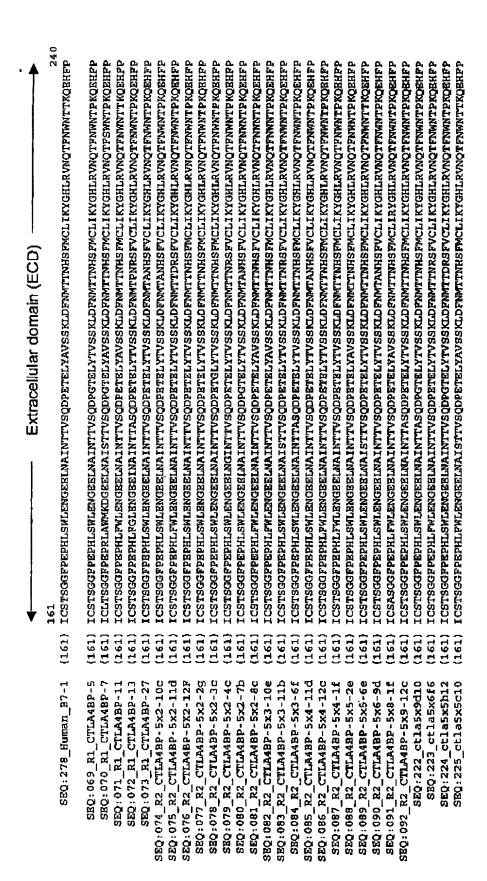


Fig. 3E

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Fig. 3F

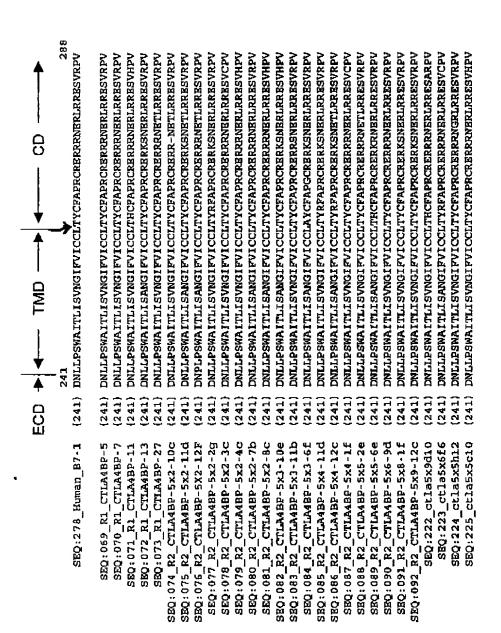


Fig. 3(

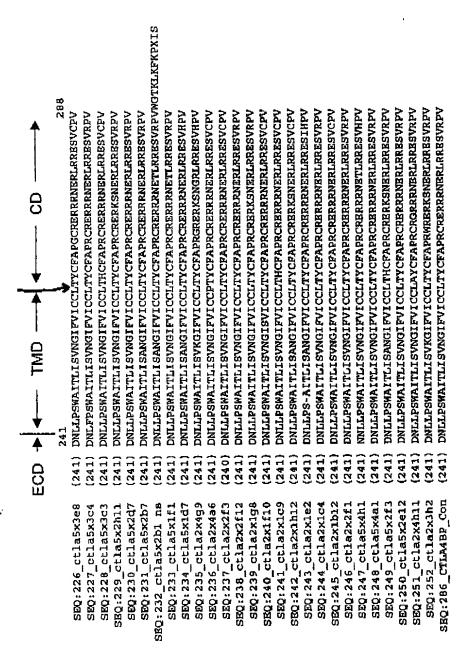
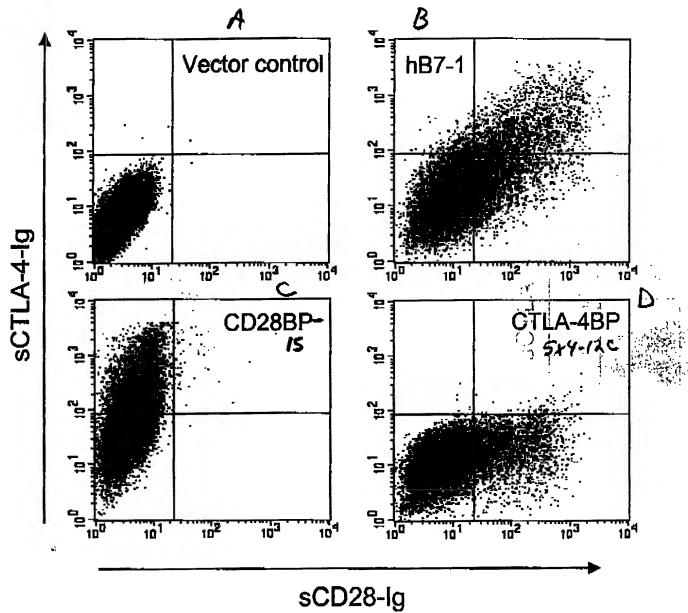
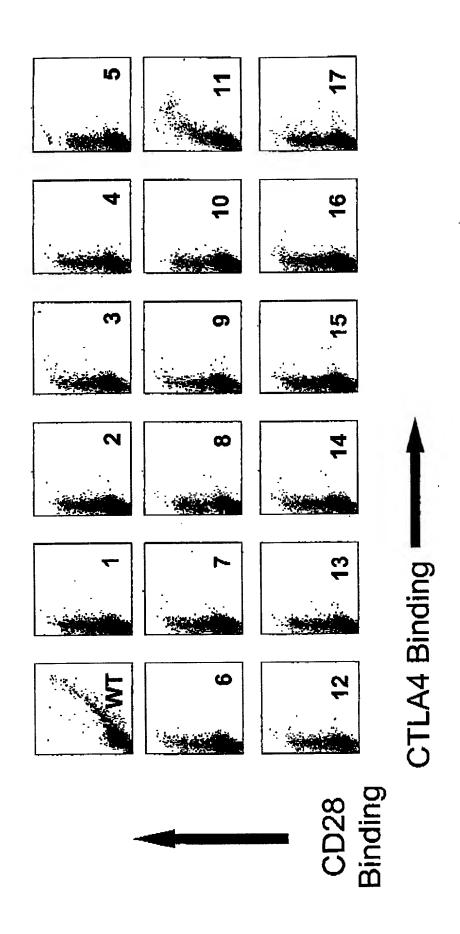


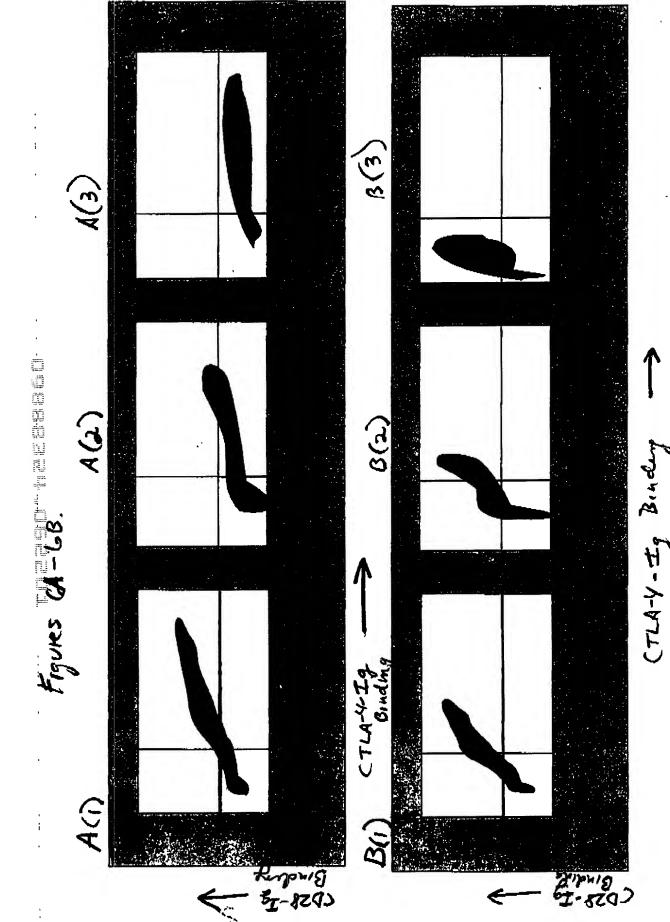
Fig. 3H



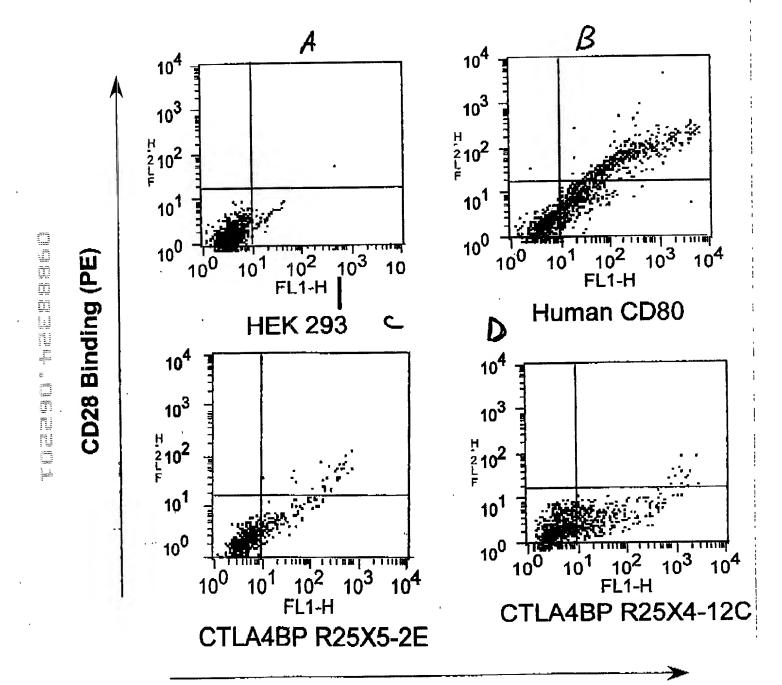
ngastala. Testil

## CD28BP after 2nd Round of Shuffling



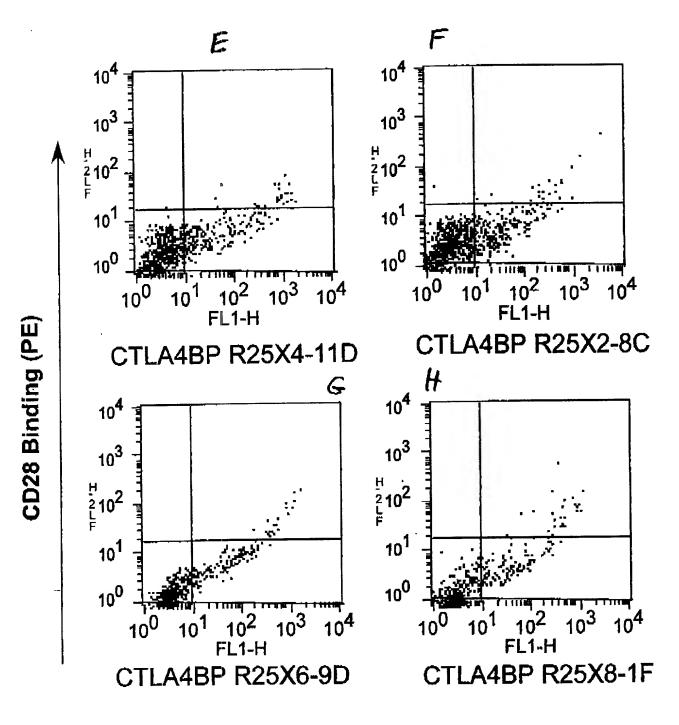


(TLA-4-If Bindy



**CTLA4 Binding (FITC)** 

#### Figures 7E-7H



**CTLA4 Binding (FITC)** 

#### CTLA-4BP - 5x4-12c Ø

**RIHWOKEKKMVLTMMSGDMNIWPEYKNRŤIFDITNNĽSIVILALRPSDEGTYECVVLKYEKDAF** LANGER LANGER KREHLAEVMLSVKADFPTPSISDFEIPPSNIRRIICSTSGGFPEPHLFWLENGEELNAINTTVSQ <u>DPETELYTVSSKLDFNMTTNHSFMCLIKYGHLRVNQTFNWNTPKQEHFPDNLLPSWAITLISA</u> <u>MGHTRRQGTSPSKCPYLKFFQLLVLAGLSHFCSGVIHVTKEVKEVATLSCGHNVSVEELAQT</u> -hesus/baboon \* ->1- Suboon HESUS NGIEVICCL TYRFAPRCRERKSNETLRRESVRPV orangutan baboon - Noman -A homen SAPI M rhesus/baboons

#### CD28BP-15

GAÝKĽEHLAŠVŘLŇÍŘADFPVPŤIŇDĽĞŇPŠĎNIRRĽICSTSGGFPŘPHLÝWLENGEELNAŤNT

CO O A Francist COO

TVSQDPĞTELYMİSSĚLDFNÝTŇNHSÍVCLIKYGĚLŠVŠOĬFĎWŠŘĎKOEĎPÍĎČLPĚWVIIPVŠ

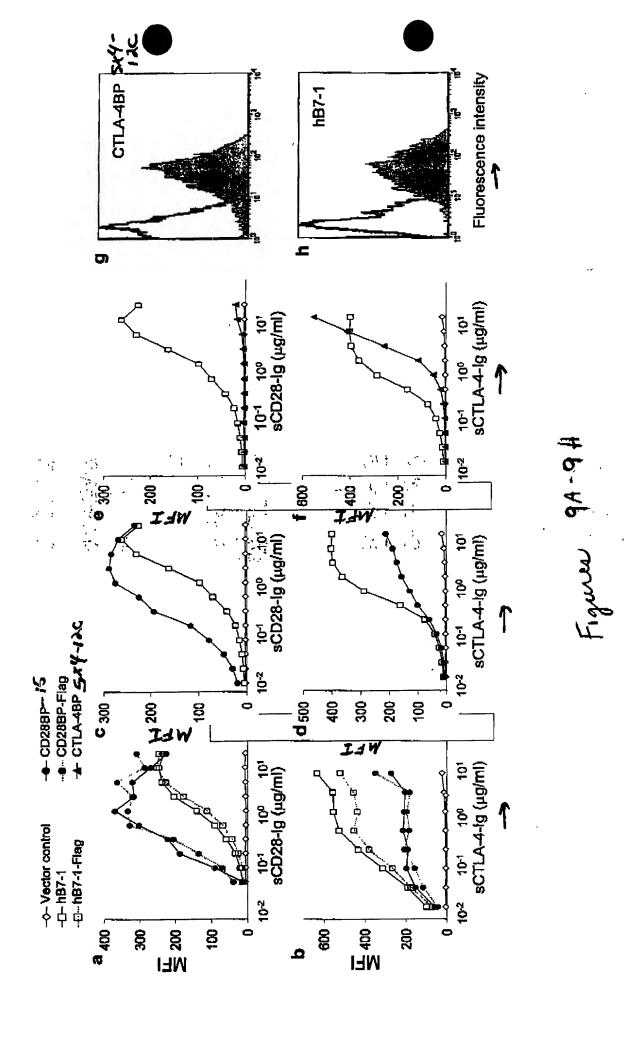
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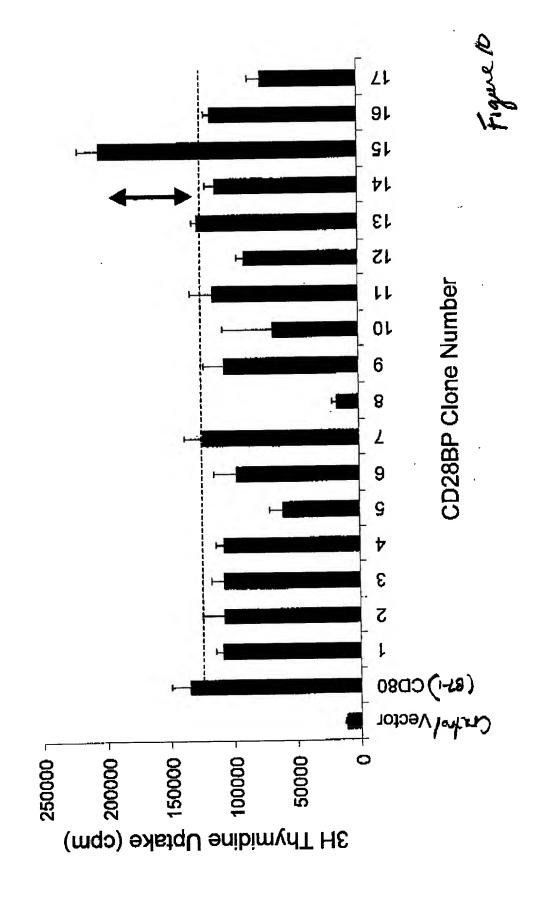
rhesus/baboon orangutan baboon human rhesus

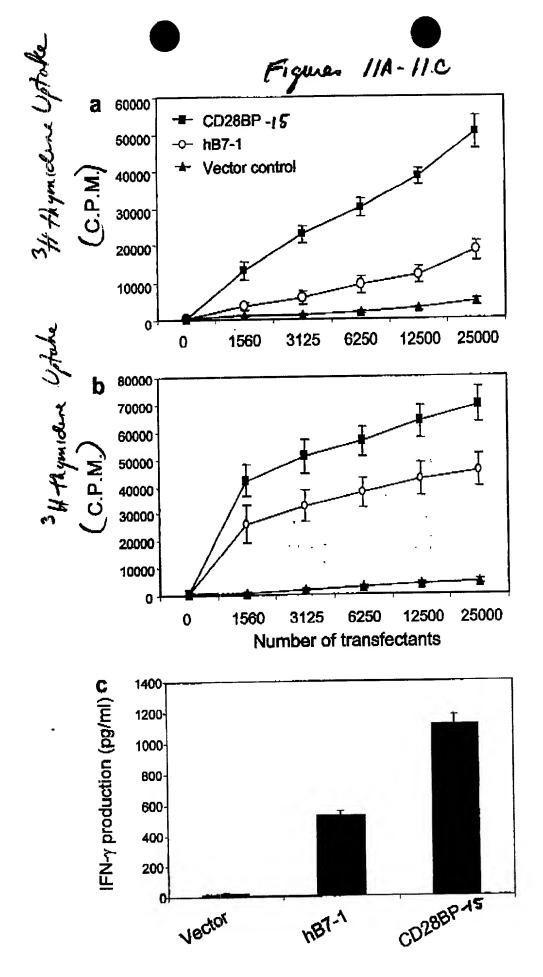
K- ナララマイ

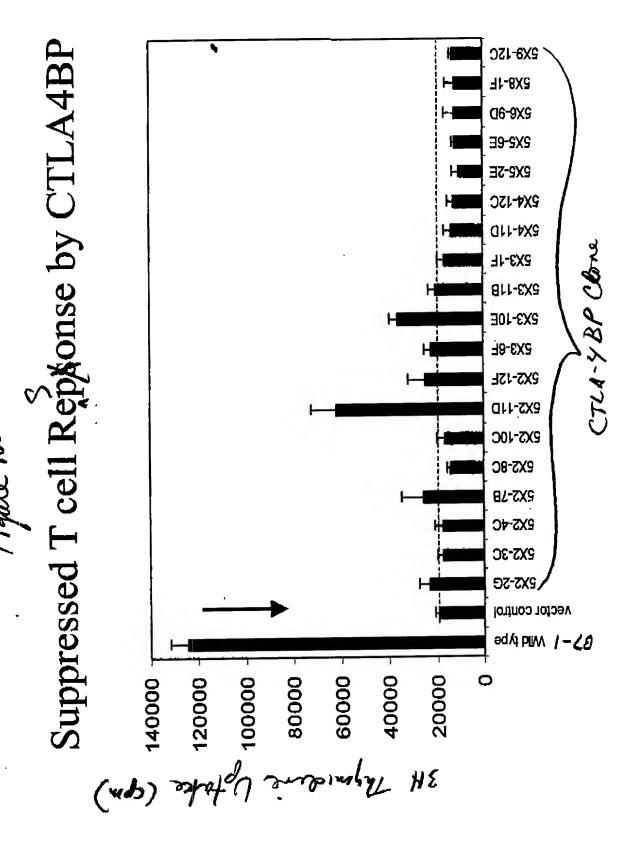
rabbit **₹**00

300









Figures 13 A-13D 34 thymidene Upterle C.P.M. (x103) B **b** 50 40 40 30 30 20 20 10 10 CTLAMBP 2C 0 CD28BP45 0 Vector 1 MBT-1 3125 6250 12500 25000 1562 Number of transfectants IL-10 production (ng/ml) **Q** 1995EP+ LEFELT IFN-y production (ng/ml) Ω 2.5 3.5 3.0 2.0 2.5 1.5 2.0 1.5 1.0 1.0 0.5 0.5 nector of 0 negaral 0 WT hET-1

#### Soluble Forms

4

Human B7.1 sECD

AAAGAPVPYPDPLEPPR AAHHHHHH

VIH.....TTKQEHFPDN

Extracellular Domain

Signal

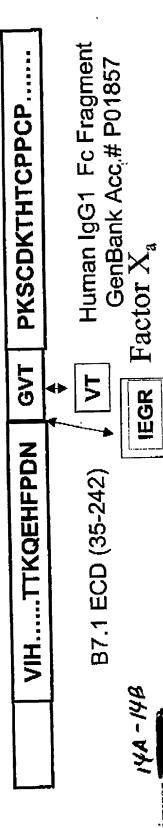
(35-242)

(1-34)

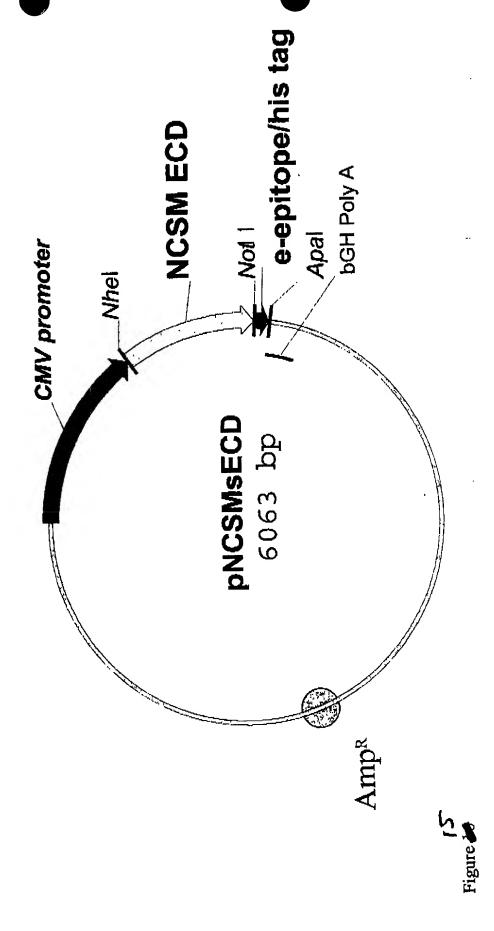
E-epitope His-tag ( 243-259 ) (260-268)

B Human B7.1 ECD-lg Form

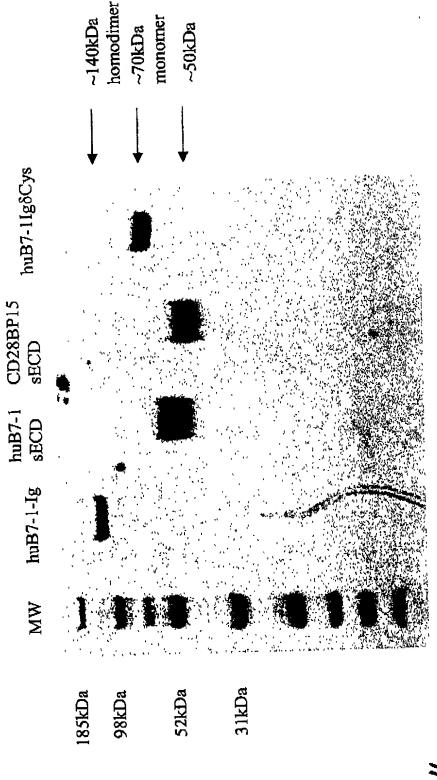
Bstell hinge -CH2-CH3



### NCSM-sECD Expression Construct

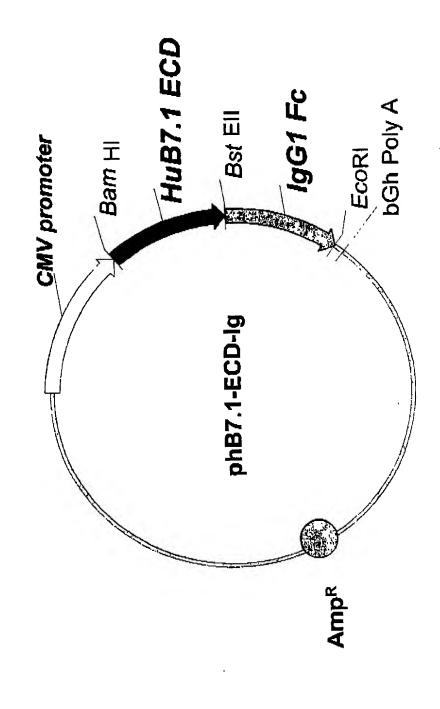


SDS-PAGE showing various soluble forms of wt & NCSM proteins



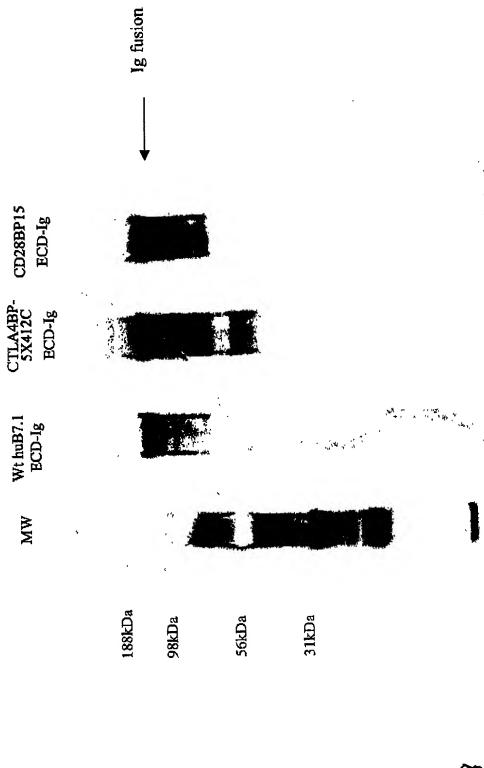


B7-1-ECD-Ig Fusion Expression Construct





Scale-up Production of wild-type soluble Human B7.1-, CTLA4BP 5X4-12C-, and CD28BP-15 ECD-Ig Fusion Proteins





# Expression of CTLA-4-BP-Ig and CD28-BP-Ig Proteins

WW CTLA4-5X2-8C ECD-Fc CTLA4-5X4-11D ECD-Fc CTLA4-5X4-12C ECD-Fc CD58Bb-8 ECD-Lc CD38Bb-11 ECD-Lc CDS8Bb-12 ECD-Lc CTLA-45X5-2E ECD-Fc CTLA-5X6-9D ECD-Fc CTLA4-5X8-1F ECD-Fc

Vector

54-f.78uf



~140 kDa



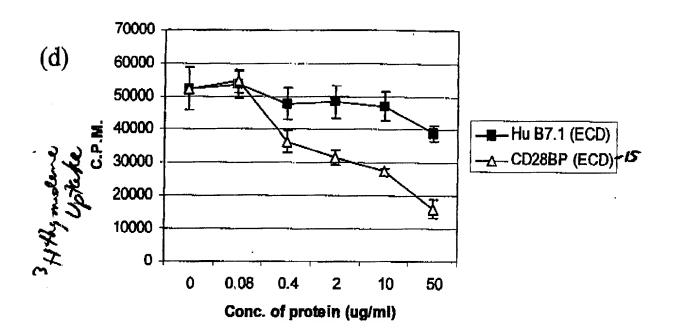


Figure 20D

